Li et al. Supplemental Data

Supplemental Figures and Legends

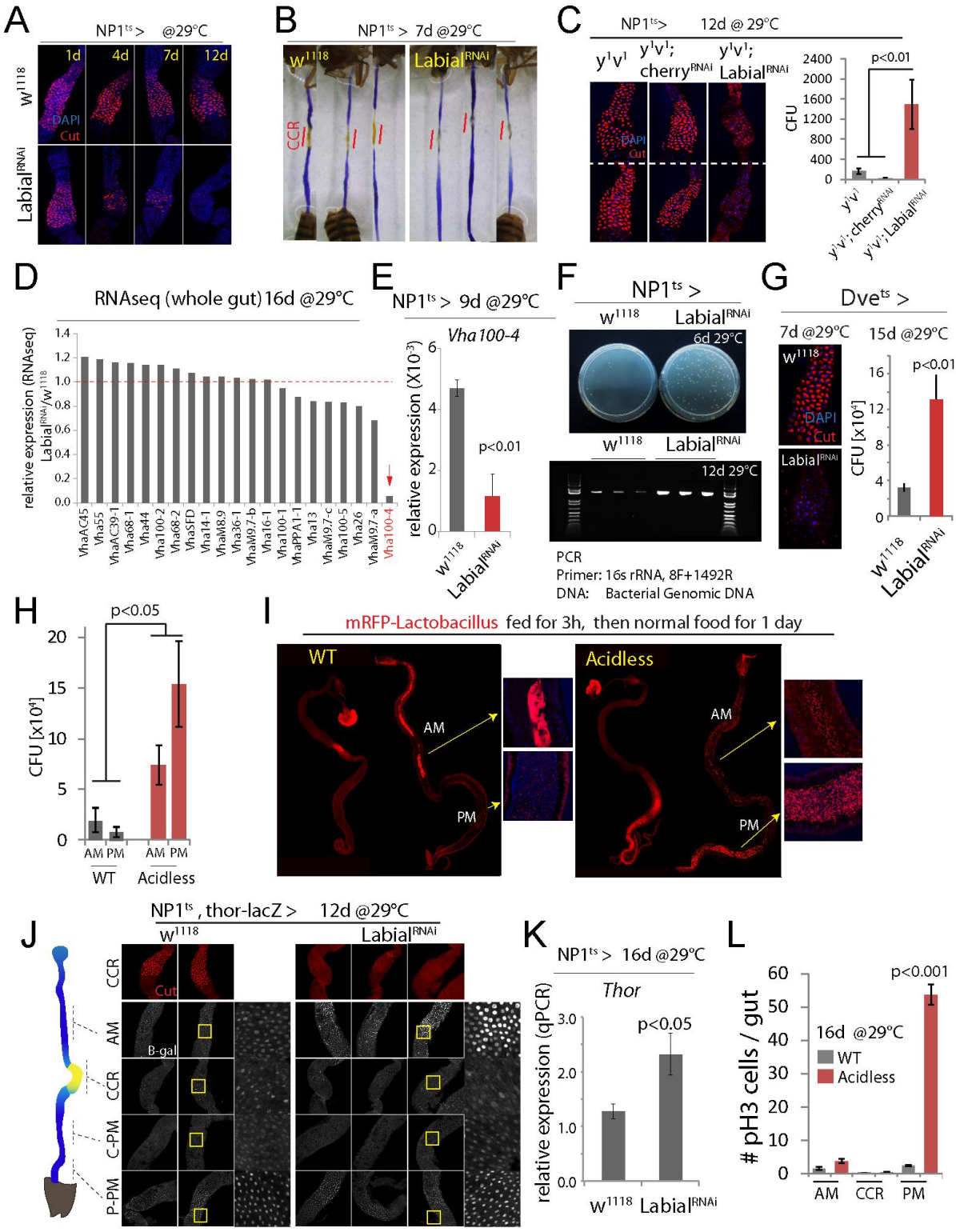


Figure S1. Intestinal compartmentalization impacts gut microbiota (related to Figure 1)

(A) Expression of Labial^{RNAi} driven by NP1::Gal4^{ts} results in progressive loss of Cut+ (red) CCs. DAPI blue. Representative images of 7 analyzed flies for each genotype and time point.

(B) GI tracts of flies fed Bromophenol blue. Acidic regions (yellow) indicate the position of the CCR. Note loss of acidity in Labial^{RNAi} expressing GI tracts.

(C) Expression of Labial^{RNAi} driven by NP1::Gal4^{ts} leads to CCR loss and significantly higher numbers of CFUs (colony forming units), while control flies (y^1v^1, or y^1v^1 mcherry^{RNAi} with the same genetic background of Labial^{RNAi}) have normal CCRs and low CFUs. Averages and s.e.m. (t-test). N=10 each.

(D) RNAseq data from NP1^{ts}>Labial^{RNAi} intestines. FPKM values normalized to wild-type controls (NP1^{ts}>*w*^{1118}). 21 different vacuolar H+ ATPases can be detected, and specific loss of the CCR-specific *Vha100-4* is notable. Averages of two samples for each genotype are shown.

(E) qRT-PCR confirming reduced *Vha100-4* expression in NP1^{ts}>Labial^{RNAi} flies. Averages and s.e.m. (t-test) of N=3 biological replicates.

(F) Gut commensals in acidless flies (NP1^{ts}> Labial^{RNAi}) or WT controls (NP1^{ts}> *w*^{1118}), measured by culturing gut extracts on nutrient rich agar plates (upper panel) or by PCR using 16S ribosomal DNA universal primers (lower panel).

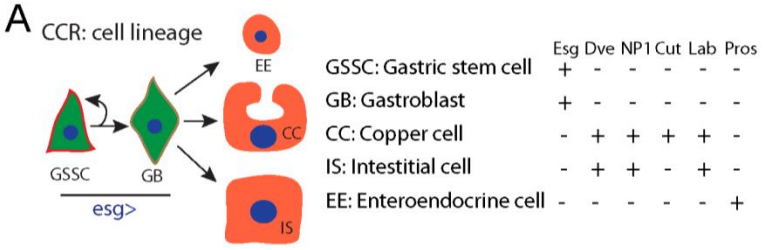
(G) Labial knockdown (Labial^{RNAi}) driven by Dve::Gal4^{ts} also results in Cut+ CC loss (red) and gut commensal expansion. CFU: colony forming units. N=10 each.

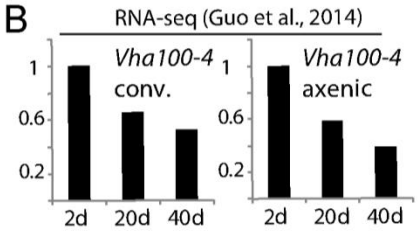
(H) Commensal distribution in 16d old WT and acidless guts. AMs and PMs were manually dissected, homogenized, and plated on nutrient rich plates. Colony-forming unit (CFU) numbers in the AM and PM are shown. WT N=12, acidless N=11. Averages and s.e.m. (t-test).

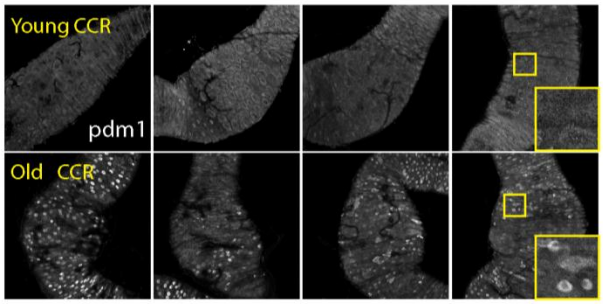
(I) Representative images of 16d old WT and acidless guts with RFP-tagged Lactobacillus. Flies were fed RFP-tagged Lactobacillus for 3 hours, and then transferred to normal food for 1d before dissection.

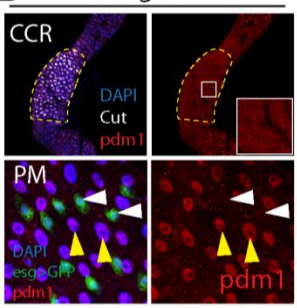
(J, K) Acidless flies (NP1^{ts}>Labial^{RNAi}) show increased Foxo activity along the GI tract, as indicated by the Foxo reporter Thor::lacZ (J) and by increased *Thor* expression detected by qRT-PCR (K). Averages and s.e.m. (t-test) of N=3 biological replicates. Cut, red, b-Gal, white. P-PM: posterior PM. C-PM: central PM. Images representative of 5 flies for each genotype.

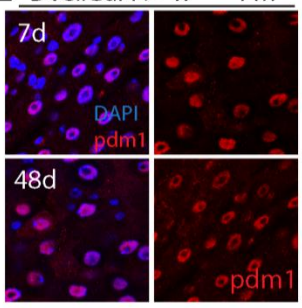
(L) Quantification of mitotic figures (pH3+ cells) in different gut regions of WT (N=12) and acidless (N=11) flies 16d after shift to 29 ℃. Averages and s.e.m. (t-test).











F

25°C	5d WT			50d WT			Student's t-test
Sample #	#1	#2	#3	#1	#2	#3	5dWT/50dWT
Lactobacillus fructivorans	0.39	0.40	0.27	0.00	0.00	0.08	**
Lactobacillus homohiochii	0.40	0.36	0.28	0.00	0.00	0.08	**
Lactobacillus senmaizukei	0.18	0.19	0.14	0.01	0.05	0.07	**
Leuconostoc pseudomesenteroides	0.00	0.00	0.00	0.56	0.46	0.39	***
Leuconostoc palmae	0.00	0.00	0.00	0.22	0.15	0.14	**
Leuconostoc carnosum	0.00	0.00	0.00	0.12	0.09	0.08	***
Acetobacter pasteurianus	0.00	0.03	0.21	0.01	0.02	0.01	ns
Lactobacillus brevis	0.00	0.00	0.00	0.01	0.08	0.04	ns
Lactobacillus acidifarinae	0.01	0.01	0.01	0.01	0.05	0.03	ns
Fructobacillus pseudoficulneus	0.00	0.00	0.00	0.02	0.01	0.01	***
Lactobacillus parabrevis	0.00	0.00	0.00	0.00	0.03	0.01	*
Methylobacterium komagatae	0.01	0.00	0.03	0.00	0.00	0.00	ns
Acetobacter oeni	0.00	0.00	0.00	0.01	0.02	0.00	ns
Acetobacter aceti	0.00	0.00	0.00	0.01	0.02	0.00	ns
Candidatus Blochmannia rufipes	0.00	0.00	0.01	0.00	0.00	0.00	*
Total	**0.99**	**0.99**	**0.94**	**0.99**	**0.99**	**0.97**	

G

																	One-way ANOVA		
29°C	5d WT				16 WT				35 WT				16d Acidless				16dWT/	16dWT/	35dWT/
Sample #	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	35dWT	16dAl	16dAl
Acetobacter pasteurianus	2.08	3.22	7.95	2.73	43.28	85.10	53.04	84.81	50.10	54.64	53.03	66.94	26.87	37.48	30.78	64.01	ns	ns	ns
Lactobacillus fructivorans	0.74	3.07	1.58	5.87	15.43	0.22	0.28	0.50	24.06	21.61	23.06	14.17	34.92	31.50	35.30	14.34	*	**	ns
Lactobacillus homohiochii	0.23	1.76	0.53	3.28	9.26	0.13	0.14	0.24	14.07	12.39	12.07	9.12	21.09	16.53	18.16	7.42	*	**	ns
Lactobacillus senmaizukei	1.22	2.32	1.68	3.96	4.51	0.21	0.46	0.27	6.98	6.13	6.92	4.04	10.23	9.54	10.80	5.01	*	**	ns
Stenotrophomonas pavanii	4.61	2.52	8.25	7.74	1.25	0.52	1.88	0.60	0.01	0.00	0.00	0.01	0.05	0.01	0.01	0.01	**	**	ns
Hydrogenophaga defluvii	1.08	10.98	3.75	3.49	0.01	0.00	1.66	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	ns	ns	ns
Escherichia albertii	1.05	2.47	1.31	1.32	3.26	1.49	7.00	0.70	0.00	0.00	0.00	0.00	0.30	0.04	0.14	0.10	ns	ns	ns
Escherichia fergusonii	0.59	1.96	0.62	0.47	1.66	0.72	3.40	0.41	0.00	0.00	0.00	0.00	0.14	0.03	0.06	0.06	*	ns	ns
Methylobacterium jeotgali	6.24	4.63	1.71	0.41	0.86	0.07	1.21	0.29	0.01	0.00	0.01	0.00	0.06	0.02	0.03	0.00	*	ns	ns
Stenotrophomonas maltophi	2.44	1.51	5.22	3.72	0.58	0.32	0.86	0.34	0.01	0.00	0.00	0.00	0.05	0.01	0.00	0.00	**	**	ns
Agrobacterium viscosum	3.65	1.16	5.95	2.67	0.21	0.00	0.67	0.16	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	ns	ns	ns
Agrobacterium tumefaciens	3.61	1.26	5.03	3.43	0.22	0.01	0.69	0.07	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	ns	ns	ns
Delftia lacustris	1.13	3.27	2.79	4.05	0.46	0.01	0.97	0.43	0.00	0.00	0.00	0.00	0.05	0.02	0.02	0.00	*	ns	ns
Acetobacter lambici	0.14	0.25	0.26	0.21	0.74	1.34	0.96	1.62	0.73	0.90	0.77	1.05	0.37	0.68	0.43	0.97	ns	ns	ns
Serratia entomophila	0.51	1.61	0.66	0.67	1.71	0.71	3.68	0.30	0.00	0.00	0.00	0.00	0.16	0.02	0.07	0.06	ns	ns	ns
total	**29.32**	**41.99**	**47.29**	**44.02**	**83.44**	**90.85**	**76.90**	**90.74**	**95.97**	**95.68**	**95.86**	**95.33**	**94.32**	**95.88**	**95.81**	**91.98**			

Figure S2. Age-related gastric decline and metaplasia (related to Figure 2)

(A) Markers and drivers specific for different cell types in the CCR. esg::Gal4, Dve::Gal4 and NP1::Gal4 are Gal4 drivers. Anti-Cut, anti-Labial and anti-prospero antibodies are used to identify individual cell types.

(B) The expression of CCR-specific Vacuolar-type H+ ATPase, Vha100-4, decreases with age in both conventional and axenic conditions. Data is from RNAseq experiments described in (Guo et al, 2014), and FPKM values at 2d are normalized to 1.

(C) Pdm1 (white) is not expressed in 3d young CCRs, but Pdm1+ cells are observed in 48d old CCRs, of w^{1118} flies.

(D) Pdm1 expression pattern in the gut of young flies (CCR outlined; Cut white, DAPI blue, Pdm1 red). Note that Pmd1 is not expressed in the CCR, but in ECs of the anterior midgut (AM) and posterior midgut (PM). Note that only one representative image of PM is shown. White arrowhead: esg+ ISC/EB, yellow arrowhead: esg- polyploid ECs. Images representative of 7 flies analyzed at each age.

(E) Pdm1 expression in the PM (Pdm1 red, DAPI blue) does not change between 7d and 48d old flies. Images representative of 7 flies analyzed at each age.

(F, G) Percentages of top 15 bacterial species from 5d and 50d old w^{1118} flies at 25 ℃ (F; 3 samples, 10 guts each sample), and from 5d, 16d, and 35d old wildtype ($NP1^{ts}>w^{1118}$) and 16d old acidless flies ($NP1^{ts}>Labial^{RNAi}$) at 29 ℃ (G; 4 samples, 10 guts each sample). Bacterial species are determined by 16S rRNA sequencing. Student's t-test and one-way ANOVA Tukey test are applied. *P<0.05; **P<0.01; ***P<0.001.

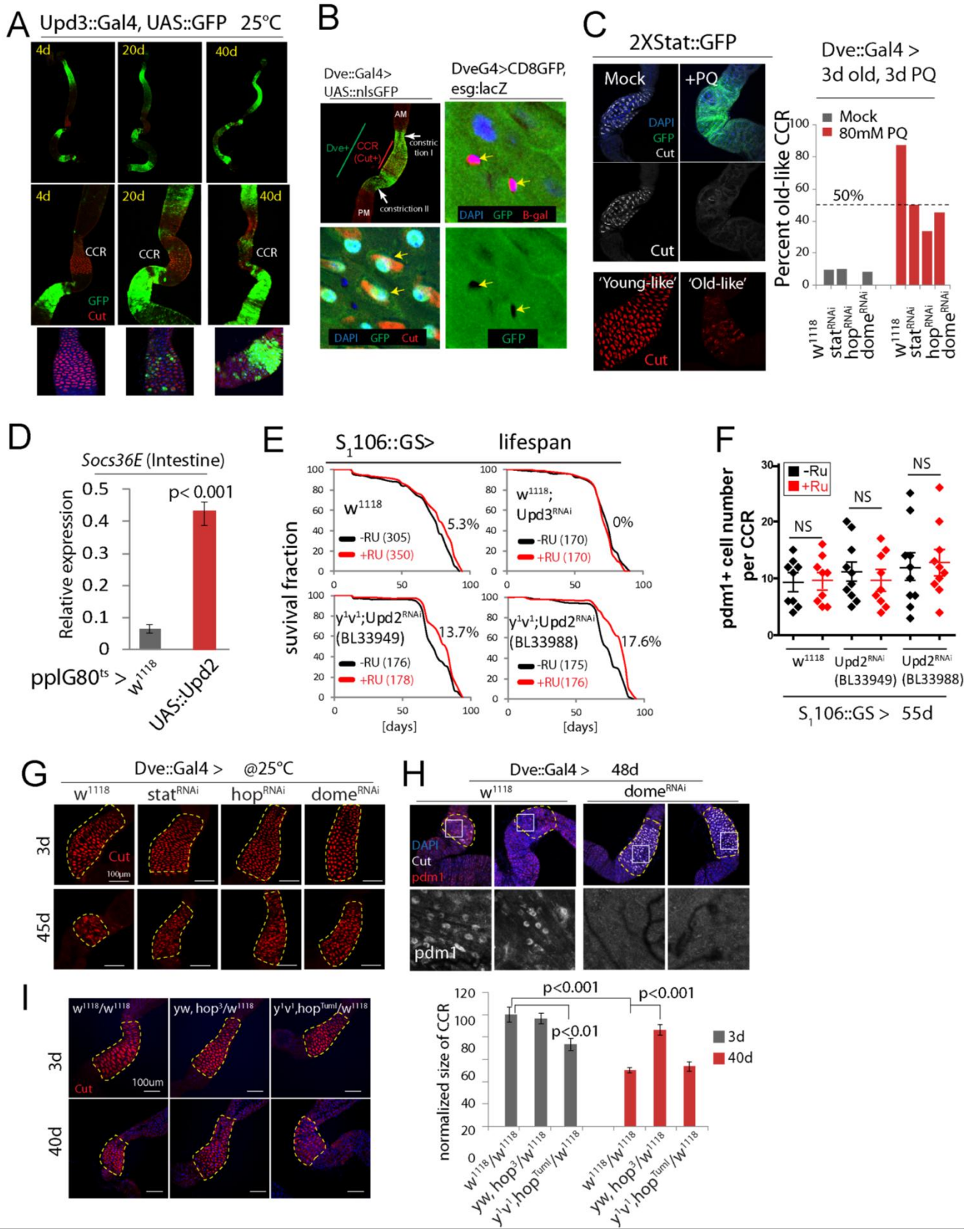

A
Upd3::Gal4, UAS::GFP 25°C
4d
20d
40d
CCR
GFP
Cut
B
Dve::Gal4>
UAS::nlsGFP
DveG4>CD8GFP,
esg:lacZ
AM
PM
Dve+
CCR
(Cut+)
constriction I
constriction II
DAPI GFP B-gal
DAPI GFP Cut
GFP
C
2XStat::GFP
Mock
+PQ
DAPI
GFP
Cut
'Young-like'
'Old-like'
Dve::Gal4 >
3d old, 3d PQ
Mock
80mM PQ
Percent old-like CCR
50%
w1118
statRNAi
hopRNAi
domeRNAi
D
Socs36E (Intestine)
p< 0.001
Relative expression
pplG80ts > w1118
UAS::Upd2
E
S1106::GS>
lifespan
suvival fraction
w1118
-RU (305)
+RU (350)
5.3%
w1118;
Upd3RNAi
-RU (170)
+RU (170)
0%
y1v1;Upd2RNAi
(BL33949)
-RU (176)
+RU (178)
13.7%
y1v1;Upd2RNAi
(BL33988)
-RU (175)
+RU(176)
17.6%
[days]
F
-Ru
+Ru
pdm1+ cell number
per CCR
NS
w1118
Upd2RNAi
(BL33949)
Upd2RNAi
(BL33988)
S1106::GS > 55d
G
Dve::Gal4 > @25°C
w1118
statRNAi
hopRNAi
domeRNAi
3d
45d
Cut
100um
H
Dve::Gal4 > 48d
w1118
domeRNAi
DAPI
Cut
pdm1
I
w1118/w1118
yw, hop3/w1118
y1v1,hopTuml/w1118
3d
40d
Cut
100um
normalized size of CCR
p<0.001
p<0.01
3d
40d

Figure S3. Age-related activation of JAK/Stat causes CC loss and metaplasia (related to Figure 3)

(A) Expression of upd3::Gal4 (UAS::GFP) in the whole gut (top panels) and CCR (lower panels) (Cut red, GFP green, DAPI blue). Images representative of 7 flies for each group at each age.

(B) Dve::Gal4 (UAS::GFP) expression in the intestine. Dve::Gal4 is active in differentiated cells of the CCR, extending somewhat into the AM and PM. The CCR is delimited by two constrictions on both sides, constriction I and constriction II. For all figures in this paper, the extent of the CCR is determined by either anti-Cut staining or by Dve::Gal4, UAS::GFP taking into account the two constrictions. Within the CCR, Dve::Gal4 is active in polyploid differentiated cells, including Cut+ CCs, but not in diploid esg::lacZ+ GSSCs/GBs. GFP green, DAPI blue in all panels, Cut red in panels on the left, B-Gal red in panels on the right.

(C) Treatment with 80mM Paraquat (PQ) for 3 days activates JAK/Stat (2x Stat::GFP) in the CCR and causes Cut+ CC loss. CCRs can be divided into two groups by anti-Cut staining ('Young-like' and 'Old-like', quantification on the right). Percent 'old-like' CCRs of (left to right) N=32, 20, 10, 24, 31, 24, 15, 31 analyzed samples. Data representative of 2 independent experiments.

(D) qRT-PCR analysis of *Socs36E* expression in the gut upon Upd2 over-expression from the fat body using ppl::G80^{ts}. Averages and s.e.m. (t-test) of N=3 biological replicates.

(E) Demographies of flies expressing Upd3^{RNAi} and Upd2^{RNAi} (two lines) under the control of fatbody and gut specific driver $S_1$106::GS (*w*^{1118} as control). All flies are female, and fly numbers and percent changes of median lifespan are indicated.

(F) Quantification of Pdm1+ cells per CCR in 55d old flies from different conditions as indicated. Averages and s.e.m. (t-test). N=9, 9, 10, 9, 10, 10.

(G) CCRs of 3d or 45d old WT flies (Dve::Gal4> *w*^{1118}), or flies in which JAK/Stat components were knocked down (Dve::Gal4> Stat^{RNAi}, Hop^{RNAi}, or Dome^{RNAi}). The CCRs are outlined (yellow) and their sizes are quantified in fig. 2d. Cut red, DAPI blue. Scale bars are indicated. Representative images of (3d – left to right) 12, 11, 11, 12 flies, or (45d) 20, 20, 16, 22 flies.

(H) Pdm1 expression in 48d old CCRs of WT flies (Dve::Gal4> *w*^{1118}) or flies expressing Dome^{RNAi} in CCR (Dve::Gal4> Dome^{RNAi}). CCRs are identified by anti-Cut and constrictions as described above (outlined in yellow). Top panels: Cut white, Pdm1 red, DAPI blue. Lower panels: Pdm1 white. Representative images of 17 flies (WT) or 22 flies (Dome^{RNAi}).

(I) Representative images of young and old CCRs from *w*^{1118} (+/+), *hop*^{3}, or *hop*^{Tuml} heterozygotes (hop^{3}/+, hop^{tuml}/+). The CCRs are outlined and their sizes are quantified on the right. Cut red, DAPI blue. Scale bars are indicated. Averages and s.e.m. (t-test). Left to right: N=13, 11, 11, 15, 10, 13. Data are representative from 2 independent experiments.

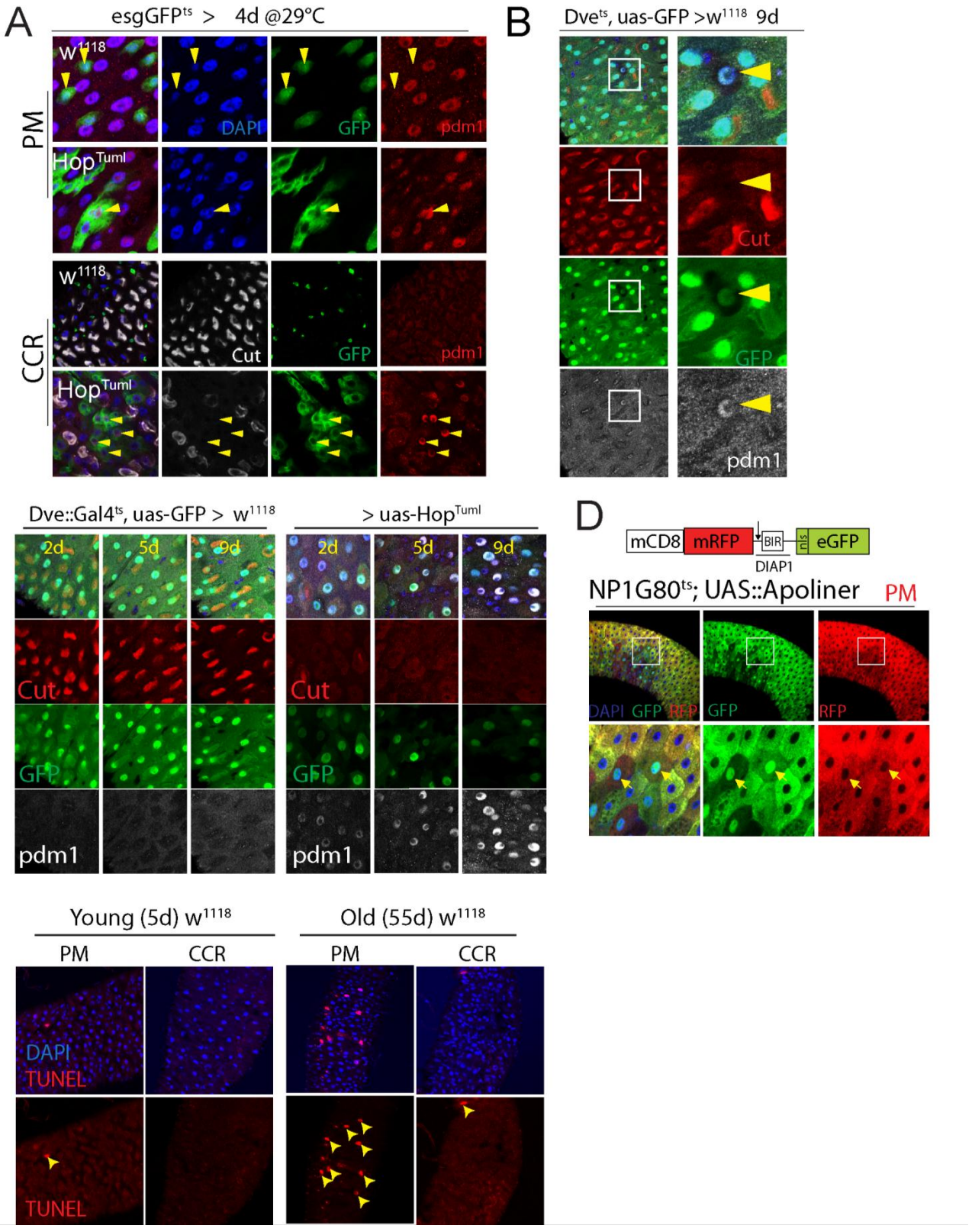

A
esgGFP^ts > 4d @29°C
w1118
DAPI
GFP
pdm1
PM
HopTuml
CCR
Cut
B
Dve^ts, uas-GFP >w1118 9d
C
Dve::Gal4^ts, uas-GFP > w1118
> uas-HopTuml
2d
5d
9d
D
mCD8
mRFP
BIR
nls
eGFP
DIAP1
NP1G80^ts; UAS::Apoliner
PM
E
Young (5d) w1118
Old (55d) w1118
TUNEL

Figure S4. Mechanisms of metaplasia: GSSC mis-differentiation and CC tran-differentiation (related to Figure 4)

(A) Over-expression of Hop^{tuml} in SCs induces Pdm1+ cells in the PM and CCR. esg::Gal4 is expressed in ISCs/EBs of the PM and in GSSCs/GBs in the CCR. In WT PM, Pdm1 is expressed in ECs of the PM (yellow arrowheads) but not in GFP+ SCs/EBs; in WT CCR, no Pdm1 expression is observed. Hop^{TumL} over-expression causes accumulation of Pdm1+ large GFP+ cells (yellow arrowheads), both in the PM and the CCR. Representative images of 7 analyzed flies for each genotype.

(B) Representative images of sporadic Pdm1+ cells in 9d old WT ($Dve^{ts}>w^{1118}$) CCRs (maintained at 29℃). Note that the Pdm1+ cell (yellow arrowhead) shows weaker Dve::Gal4> nlsGFP compared to surrounding Pdm1- cells.

(C) Progressive increase of Pdm1 expression (white) and loss of Cut+ CCs (red) in CCRs over-expressing Hop^{Tuml} under the control of $Dve::Gal4^{ts}$. Note also the progressive decline in GFP (green) expression in Hop^{Tuml} expressing cells, indicating reduced activity of the Dve::Gal4 driver. Images representative of N=6 flies for each genotype and time point.

(D) Assessment of apoptosis using Apoliner (Bardet et al., 2008). Apoliner consists of RFP and nls-GFP separated by a Caspase – sensitive sequence from Diap1 and tethered to the cell membrane by CD8. Upon Caspase activation, the Diap1 fragment is cleaved, allowing nls-GFP to translocate to the nucleus. Representative images of a wild-type PM are shown indicating that apoptotic cells (arrowheads) can readily be detected by Apoliner (construct is expressed using $NP1^{ts}$).

(E) Representative images of TUNEL staining (red, arrowhead) in PMs and CCRs of young and old flies.

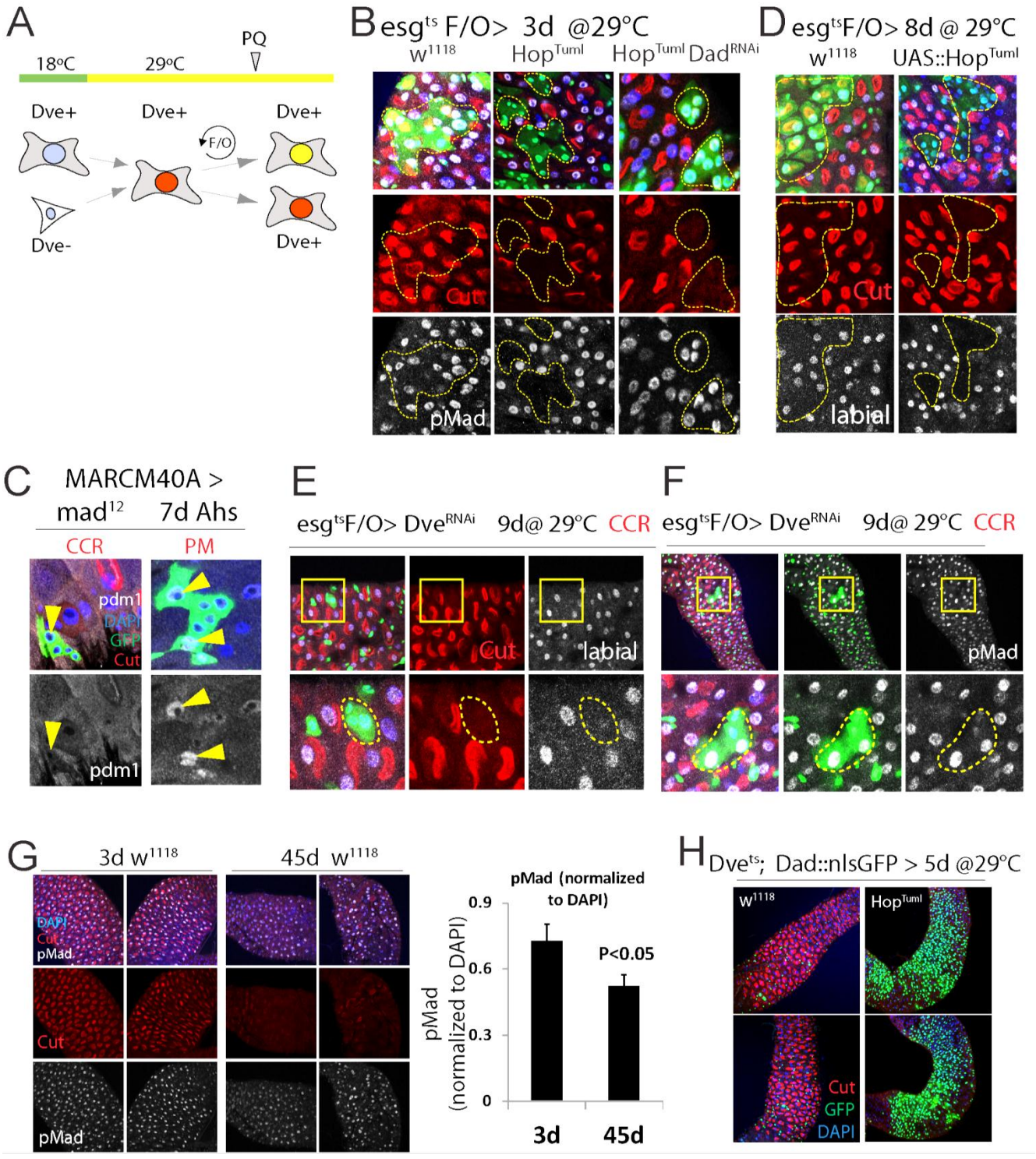


Figure S5. Mechanisms of metaplasia: GSSC mis-differentiation and CC tran-differentiation (related to Figure 4)

(A) Schematic of G-Trace experiment. Genotype is Dve::Gal4^{ts}, UAS::RFP, UAS::FLP, Ubi-STOP-GFP. At 18 ℃, Dve::Gal4 is repressed by tub::Gal80^{ts}. When transferred to 29 ℃, Dve::Gal4 will drive UAS::RFP and UAS::Flp in the CCR, flipping out (F/O) the STOP cassette between the ubiquitin promoter (Ubi) and GFP. 'Flipped' cells will express RFP and GFP, appearing yellow. 'Un-flipped' cells will only express RFP. 24h Paraquat (PQ) treatment was performed 3d after the flies were transferred to 29 ℃.

(B) GSSC clones (outlined, esg^{ts}F/O>), over-expressing Hop^{Tuml} do not contain Cut+ CCs (red) and have lower anti-pMad staining (white) compared to surrounding cells. Knockdown of Dad by Dad^{RNAi} rescues Mad phosphorylation, but not the loss of Cut+ CCs.

(C) MARCM clones (green) homozygous for *mad*^{12} in the PM and CCR. Pdm1+ cells are readily detected in these clones in the PM, but not in the CCR (Cut red, DAPI blue, Pdm1 white). Representative images of 9 analyzed flies for each genotype.

(D) GSSC clones (outlined, esg^{ts}F/O>), over-expressing Hop^{Tuml} do not have anti-Labial (white) or anti-Cut (red) staining compared to surrounding cells.

(E) Dve knockdown (Dve^{RNAi}) in GSSC clones (outlined, esg^{ts}F/O>) results in loss of Cut+ CCs (red) and loss of anti-Labial staining (white).

(F) Dve knockdown (Dve^{RNAi}) in GSSC clones (outlined, esg^{ts}F/O>) does not result in reduced anti-pMad staining (white) compared to surrounding cells.

(G) Representative images of pMad antibody staining (white) in CCRs of young and old w^{1118} flies (left) and quantification of pMad intensity (normalized to DAPI, right). Averages and s.e.m. (t-test). 3d N=443 cells from 6 guts, 45d N=157 cells from 4 guts.

(H) Expression of Dad::nlsGFP in CCRs of Dve^{ts} >Hop^{Tuml} flies (w^{1118} as control).

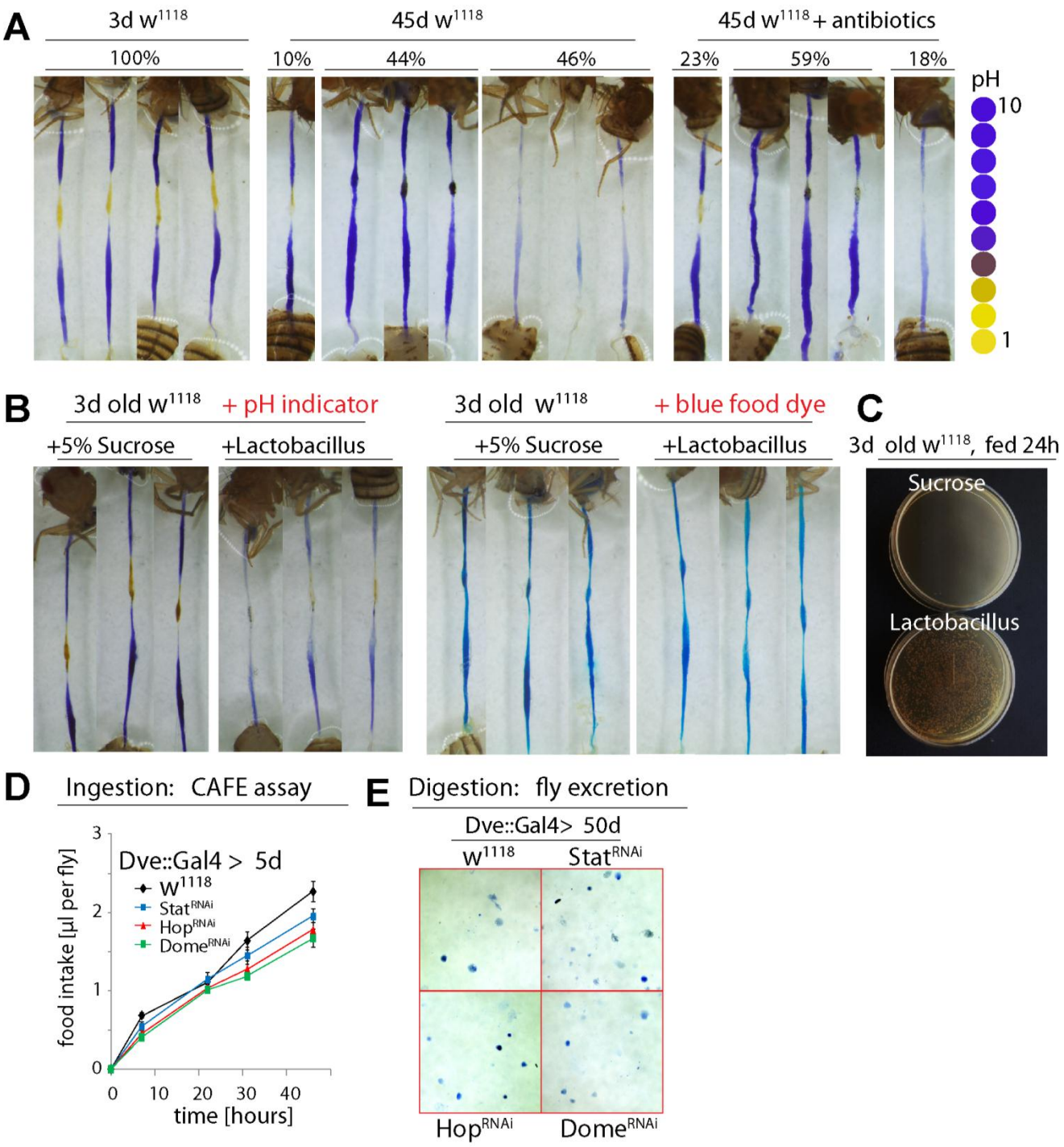


Figure S6. Inhibiting JAK/Stat in the CCR promotes gut function (related to Figure 5)

(A) Representative images of GI tracts of WT (w^{1118}) flies fed pH indicator Bromophenol Blue. Ages are indicated. Antibiotic treatment was for 4d before dissection. Images representative of N=28 (3d), 57 (45d), 39 (45d+antibiotics).

(B) Left: Representative images of GI tracts of young WT (w^{1118}) flies fed pH indicator Bromophenol Blue with *Lactobacillus* or control 5% sucrose solution. Note slight acidification of the AM and PM. Right: Representative images of GI tracts of young WT (w^{1118}) flies fed blue food dye with either sucrose or *Lactobacillus*. GI tracts look similar in color, suggesting no difference in food intake. Images representative of 10 flies for each condition.

(C) Gut extracts of young flies (w^{1118}) fed sucrose or *Lactobacillus* for 24h cultured on MRS selective plates.

(D) Food intake measured using the CAFÉ assay. Inhibiting JAK/Stat activity by expressing $Stat^{RNAi}$, Hop^{RNAi} or $Dome^{RNAi}$ in the CCR (Dve::Gal4>) does not change the food intake at 5d. Averages are shown. N=21 flies for each group (7 replicates with 3 flies each).

(E) Representative images of deposits from old (50d) flies expressing $Stat^{RNAi}$, Hop^{RNAi} or $Dome^{RNAi}$ in the CCR (Dve::Gal4) and Dve::Gal4>w^{1118} controls.

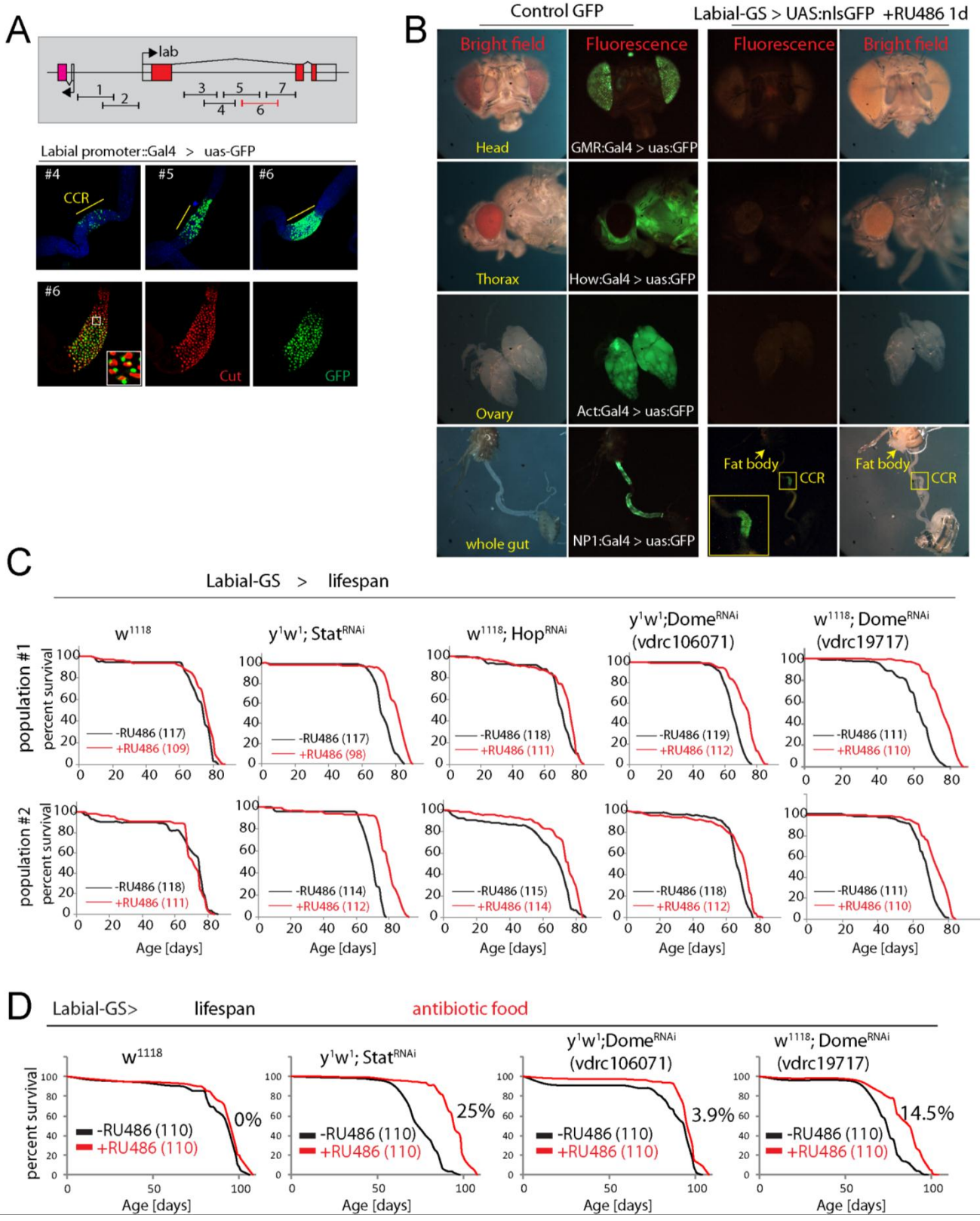

A
lab
1
2
3
4
5
6
7
Labial promoter::Gal4 > uas-GFP
#4
CCR
#5
#6
#6
Cut
GFP
B
Control GFP
Labial-GS > UAS:nlsGFP +RU486 1d
Bright field
Fluorescence
Fluorescence
Bright field
Head
GMR:Gal4 > uas:GFP
Thorax
How:Gal4 > uas:GFP
Ovary
Act:Gal4 > uas:GFP
whole gut
NP1:Gal4 > uas:GFP
Fat body
CCR
Fat body
CCR
C
Labial-GS > lifespan
w1118
y1w1; StatRNAi
w1118; HopRNAi
y1w1;DomeRNAi (vdrc106071)
w1118; DomeRNAi (vdrc19717)
population #1
percent survival
-RU486 (117)
+RU486 (109)
-RU486 (117)
+RU486 (98)
-RU486 (118)
+RU486 (111)
-RU486 (119)
+RU486 (112)
-RU486 (111)
+RU486 (110)
population #2
-RU486 (118)
+RU486 (111)
-RU486 (114)
+RU486 (112)
-RU486 (115)
+RU486 (114)
-RU486 (118)
+RU486 (112)
-RU486 (111)
+RU486 (110)
Age [days]
D
Labial-GS> lifespan antibiotic food
w1118
y1w1; StatRNAi
y1w1;DomeRNAi (vdrc106071)
w1118; DomeRNAi (vdrc19717)
percent survival
-RU486 (110)
+RU486 (110)
0%
25%
3.9%
14.5%
Age [days]

Figure S7. Inhibiting JAK/Stat in the CCR extends lifespan (related to Figure 6)

(A, B) Labial-GeneSwitch construction and validation.

(A) Construction of labial::GeneSwitch. Seven Janelia-Gal4(Jenett et al., 2012) lines with different Labial promoter regions (indicated on the schematic) were crossed with UAS::GFP to assess driver activity in the intestine. Three Gal4 lines (#4, #5, and #6) have GFP expression in the CCR, and #6 shows the best overlap with Cut+ cells in the CCR. The promoter region of #6 was used to construct Labial::GeneSwitch. See Methods for details.

(B) Validation of Labial::GeneSwitch. After exposure to RU486, Labial::GS activity can be detected specifically in the CCR of the intestine, but not in other regions or tissues. Established Gal4 lines are used as imaging controls: GMR::Gal4 for the eye, How::Gal4 for the thoracic muscle, Act::Gal4 for the ovary, and NP1::Gal4 for the whole intestine.

(C) Demographies of flies expressing $Stat^{RNAi}$, Hop^{RNAi} or two $Dome^{RNAi}$ lines under the control of Labial::GS. Graphs are from two independent populations of F1 progeny from crosses of Labial::GS crossed to the indicated transgenes or to WT (w^{1118}). All flies are female and Ns for each group are indicated.

(D) Demographies of flies expressing $Stat^{RNAi}$, or two $Dome^{RNAi}$ lines under the control of Labial::GS on antibiotic food, with w^{1118} as control. All flies are female, and Ns and percent changes of median lifespan are indicated.

Supplemental Experimental Procedures

Fly lines and husbandry

The following fly lines were obtained from Bloomington *Drosophila* Stock Center: w^{1118}, y^1w^1, y^1v^1, y1v1,$cherry^{RNAi}$, OreR, FRT82, $Labial^{RNAi}$ (BL26753), Dve^{RNAi} (BL26225), Dad^{RNAi} (BL33759), UAS-CD8::GFP (BL5137), How::Gal4 (BL1767), tub::$G80^{ts}$ (BL7017 and BL7108), hop^3 (BL8495), hop^{tuml} (BL8492), esg::lacZ (BL10359), UAS::Apoliner (BL32122), G-Trace (w*; UAS-RedStinger, UAS-FLP, Ubi-FRT.STOP-Stinger, BL28281), Act::Gal4 (BL4414), $Upd2^{RNAi}$ #1 (BL33949), $Upd2^{RNAi}$ #2

(BL33988). Stat^{RNAi} (vdrc106980), hop^{RNAi} (vdrc40037), dome^{RNAi} #1 (vdrc106071) and dome^{RNAi} #2 (vdrc19717, only used for Fig. 6 and Fig. S7) were obtained from VDRC stock center, Vienna. Dve::Gal4 (NP3428) from DGRC stock center, Kyoto. esg-Gal4, UAS-GFP was a gift from Shigeo Hayashi; esg^{ts}F/O (esgGal4,tubG80^{ts},UAS-GFP; UAS-flp, act>STOP>Gal4) from Huaqi Jiang; NP1::Gal4 from Dominique Ferrandon; Mad^{12},FRT40, Upd3::Gal4, and MARCM82 (hsFlp; tub-Gal4, UAS-GFP; FRT82, tubGal80) from Norbert Perrimon; GMR::Gal4 from Marek Mlodzik; 2xStat::GFP from Erika Bach; UAS::Hop^{Tuml} from David Bilder; Upd3^{RNAi} from Steven Hou; UAS-upd2 from Martin Zeidler; A142-GFP from Nicolas Buchon; esgGFP^{ts}; ppl::Gal4 from M. Pankratz; $S_1$106GS from Marc Tatar, Su(H)GBE::G80 (Wang et al., 2014).

Flies were cultured on yeast/molasses-based standard fly food (recipe: 10L H_2O, 138g agar, 220g molasses, 750g malt extract, 180 dry yeast, 800g corn flour, 100g soy flour, 62.5ml propionic acid, 20g Methyl 4-Hydroxybenzoate, and 72ml ethanol) at 25℃ with a 12h light/dark cycle. For TARGET (tubGal80^{ts}) experiments, flies were raised at 18℃ to allow Gal80 to inhibit Gal4, and 3-4 days after eclosion shifted to 29℃ to inhibit Gal80 and to allow Gal4 to drive UAS-linked transgene expression.

Flies used in this paper are all female. The reason we use females is that the female gut is best understood when it comes to stem cell regulation and epithelial regeneration. Virtually all studies on ISC function and intestinal regeneration in flies have focused on the female gut, as it is larger and has a faster turnover rate than male guts. To keep consistency with published work from us and others, we therefore used female flies for all experiments.

Immunostaining, TUNEL staining, and Microscopy

Fixative: 100 mM glutamic acid, 25mM KCl, 20 mM MgSO4, 4 mM sodium phosphate, 1 mM MgCl2, and 4% formaldehyde. Washing buffer: 1X PBS, 0.5% bovine serum albumin and 0.1% Triton X-100. Primary antibodies and dilution: rabbit anti-pSMAD3 (abcam, ab52903**;** labeled pMad in figures), 1:300; rabbit anti-β-galactosidase (Cappel), 1:5000; mouse anti-cut, anti-prospero (Developmental Studies Hybridoma Bank), 1:100, 1:250, respectively; rabbit anti-labial (gift from Thom Kaufman), 1:200; rabbit anti-Pdm1 (gift from Yu Cai), 1:300. Fluorescent secondary antibodies were from Jackson Immunoresearch. DAPI was used to stain DNA. Staining with pMad antibody was performed following the same protocol, but including a phosphatase inhibitor (Roche PhosSTOP, 1 tablet in 500ul 1XPBS as 20X stock) during fixation and primary antibody incubation.

Demography

To make a cross in one bottle, 40 virgin females and 20 males were used. Progenies ware collected 3 days after the first fly hatched and flies were allowed to mate for 2-3 days. Then female flies of indicated genotypes were put into cages (about 100 flies per cage) and aged at 25 ℃. Demographic data were analyzed using Prism statistical software.

For RU486 food, 100ul of a 5 mg/ml solution of RU486 or control (80% ethanol) was deposited on top of the food and dried for overnight to ensure complete evaporation, resulting in a 0.2 mg/ml concentration of RU486 in the food accessible to flies. For all populations, plastic cages (175ml volume, 5cm diameter from Greiner bio-one) were used for lifespan experiments. Food, changed every 2-3 days, was provided in vials inserted into a foam plug (4.9cm in diameter, 3cm thick from Greiner bio-one). Dead flies were visually identified (flies not moving, not responding to mechanical stimulation and lying on their side or back were deemed dead), and their numbers were recorded. Cages were replaced after 20 days (flies were transferred into new cages without anesthesia).

Axenic fly culture

Flies were sterilized and aged under sterile conditions as described (Guo et al., 2014). In brief, embryos were bleached for 3 min in 2.7% sodium hypochlorite [2-fold diluted bleach (Kem Tech, St. Ixonia, WI)] and then washed twice with sterile ddH_2O for 1 min. These embryos were transferred into sterile food in a tissue culture hood. Flies were maintained in a laminar flow hood and transferred every 2-3 days into new, sterile vials. Sterile food bottles and vials were generated by autoclaving at 121 ℃ for 30 min and were cooled down in the hood. To validate axenic conditions, adult fly guts were dissected and plated onto nutrient agar plates to check commensal loads.

To make antibiotic food, standard fly food was microwaved, cooled down to 50-60 ℃, mixed with a cocktail four antibiotics (final concentration 50ug/ml for each), and poured into new vials. The cocktail was composed of Ampicillin (dissolved in 50% ethanol), Tetracycline (50% ethanol), Erythromycin (50% ethanol), and Kanamycin (water). We confirmed that 3-4d culture on antibiotic food clears all gut commensals.

esg^{ts}F/O and MARCM Clone induction

Because of the intrinsic quiescence of gastric stem cells, the frequency of clone formation in the copper cell region (CCR) is very low in both the MARCM system and the esg^{ts}F/O system. Double heat-shock, however, increases the frequency of clone formation in the CCR (Strand and Micchelli, 2011). For the MARCM system, 3 day old mated female flies were heat-shocked at 37 ℃ for 45 min, allowed to recover at 25 ℃ for 2h and then heat-shocked at 37 ℃ for 45 min again. Flies were then kept at 25 ℃ for

7 days before dissection. For esg^{ts}F/O clone induction, 3 day old mated female flies (raised at 18 ℃) were shifted to 29 ℃ for 2 days, double heat-shocked, and then kept at 29 ℃ for the time indicated before being dissected.

Commensal quantification and selective plates

Selective plates were generated according to the following recipes:

Acetobacteriaceae: 25 g/l D-mannitol, 5 g/l yeast extract, 3 g/l peptone, and 15 g/l agar.

Enterobacteriaceae: 10 g/l Tryptone, 1.5 g/l yeast extract, 10 g/l glucose, 5 g/l sodium chloride, 12 g/l agar.

Lactobacilli MRS agar: 70 g/l BD Difco Lactobacilli MRS agar.

Nutrient Rich Broth: 23 g/l BD Difco Nutrient agar.

All media were autoclaved at 121 ℃ for 20 min.

RT-PCR and primer sequences

Total RNA from female whole guts or different gut regions (distinguished by the two constrictions near the CCR) was extracted using TRIzol (Invitrogen). Complementary DNA was synthesized using an oligo-dT primer. Real-time PCR was performed on a Bio-Rad CFX96 detection system. Relative expression was normalized to Actin5C.

Actin5C (F): 5’-CTCGCCACTTGCGTTTACAGT-3’

Actin5C (R): 5’-TCCATATCGTCCCAGTTGGTC-3’

Vha100-4 (F): 5’-CAGGAGAGCAACAGCATCTT-3’

Vha100-4 (R): 5’-CGTCTCACCTCGCCAATAAA-3’

Upd2 (F): 5’-ACTGTTGCATGTGGATGCTG-3’

Upd2 (R): 5’-CAGCCAAGGACGAGTTATCA-3’

Upd3 (F): 5’-ACAAGGCCAGGATCACCACCAAT-3’

Upd3 (R): 5’-TGTACAGCAGGTTGGTCAGGTTGA-3’

Socs36E (F): 5’-CAGTCAGCAATATGTTGTCG-3’

Socs36E (R): 5’-ACTTGCAGCATCGTCGCTTC -3’

Thor (F): 5’-CACTTGCGGAAGGGAGTACG-3’

Thor (R): 5’-TAGCGAACAGCCAACGGTG-3’

Dpt (F): 5’-GGCTTATCCGATGCCCGACG-3’

Dpt (R): 5’-TCTGTAGGTGTAGGTGCTTCCC-3’

RNA-Seq Analysis

Intact fly guts were dissected in PBS. Total RNA was extracted using Trizol reagent and used as template to generate cDNA libraries. The sequencing was performed on Illumina MiSeq system and expression was recorded as FPKM: fragments per kilo-base per million reads.

16S rDNA sequencing and PCA analysis

To extract commensal genomic DNA from guts, the flies were dipped into 70% ethanol for about 60 seconds to kill bacteria on the fly bodies, and were then dissected in 1X sterile PBS. The crops of fly guts were removed, but the whole midgut were left intact to avoid leakage. All the flies used are female. Each sample containing 10 female guts was processed using UltraClean Microbial DNA Isolation Kit (MO BIO). The DNA was used as templates for limited cycle PCR with primers targeting V3/V4 regions (Forward 5'-CCTACGGGNGGCWGCAG -3' and Reverse 5'-GACTACHVGGGTATCTAATCC -3') to get the 16S metagenomic sequencing library. The reaction conditions: 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 1 min, 48 ℃ for 2 min, and 72 ℃ for 2 min, and a final extension at 72 ℃ for 5 min.

Commensal DNA concentration

Gut commensal genomic DNA was extracted as described above. Then, the DNA was used as templates for limited cycle PCR with primers targeting the whole 16S rRNA gene (8F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-GGMTACCTTGTTACGACTT-3'). The reaction conditions: 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 1 min, 48 ℃ for 2 min, and 72 ℃ for 2 min, and a final extension at 72 ℃ for 5 min. DNA concentration was then measured by Epoch Microplate Spectrophotometer.

Cafe assay and fly excretion measurement

For Caf é assay, a 5ul capillary containing liquid food (10% yeast, 10% sucrose, and blue dye) was inserted into a cotton plug of 6cm long vial, and 1cm high 1% agar was at the vial bottom to keep the moisture. Three flies were in each vial. The food intake was recorded every 12h and the capillary was changed every 24h. For fly excretion measurement, flies were dry starved for 2h and put into Bromophenol blue vial food for 24h, and then the deposits on the vial wall were imaged and quantified.